within the generated model, they were not practically significant as the effect size was less than the infectivity assay variability. Temperature had a known effect on virus inactivation, since lower temperature correlated with reduced inactivation kinetics. However, this data set supported a minimal difference in inactivation due to the operation between the ranges of 15° C. and 20° C.

[0098] A prediction profiler was created to optimize the model to achieve greater than 4 LRF after both 15 and 30 minute time points as shown in FIG. **6**. FIG. **6** shows a prediction profiler representing X-MuLV inactivation as a function of the significant factors evaluated in the D-optimal DoE: red line denotes fit line; blue line denotes mean line; red shaded line denotes 95% confidence interval; P denotes phosphoric acid; and G denotes glycine HCl. With all other factors remaining constant, an increase in conductivity through the addition of NaCl at operating viral inactivation in the range of about pH 3.60 to 3.90 can result in greater retrovirus LRFs.

[0099] The testing results of the statistical DoE for low pH hold in the present application were consistent with the ASTM standard for 5.0 LRF X-MuLV inactivation at pH less than 3.60. The results also demonstrated robust and effective inactivation at pH greater than 3.60. For ranges outside the ASTM generic claim, the results indicated that increasing the NaCl content can achieve rapid and effective X-MuLV inactivation. Typically, the pH of the hold is dependent on the stability of the protein. The models of the present application can be used to predict effective clearance when operating viral inactivation in the range of pH 3.60-3.90 by manipulating conductivity of the low pH starting material.

[0100] High protein concentration, such as greater than 25 g/L, has been reported to negatively impact X-MuLV inactivation (ASTM). However, previous Regeneron studies have demonstrated that higher protein concentration can potentially improve X-MuLV inactivation kinetics under conditions where inactivation may not be complete. Conditions with increased ionic strength, such as higher buffer concentration, titration of a weak acid or higher protein concentration correlated with higher LRFs at higher pH (Chinniah et al.). Although the dataset of the present application used two monoclonal antibodies having similar concentrations, the conclusions from the data agreed with the conclusion that an increase in protein concentration would increase inactivation. More ions were added during titration due to the increase in acid titrant which was required to achieve the desired pH. The result was an increase in the ionic strength of the solution and this experiment would suggest greater inactivation kinetics.

[0101] Similar to the impact of protein concentration, there was a significant difference observed by the model for acid titrant, where the glycine HCl acid titrant (weaker acid) was correlated with higher LRF values of the phosphoric acid titrant (stronger acid). This conclusion was not practically significant because the effect size was less than 0.5 LRF. The results supported increasing inactivation due to increasing ion concentration in solution. Previous studies showed lower clearance at lower temperatures dues to the thermodynamics of virus inactivation. Temperature was statistically significant in the generated linear regression model, but had a minimal effect size within the studied range (15 to 20° C.). Previous studies conclude that there is no statistically significant difference on virus inactivation between 15° C. and 16+° C. (Mattila et al., Retrospective evaluation of low-pH virus inactivation and viral filtration data from a multiple company collaboration, PDA Journal of Pharmaceutical Science and Technology 70.3 (2016): 293-299). The experiment of the present application supported the ASTM generic viral clearance claim as well as identifying a solution to achieve effective inactivation of retrovirus at greater than pH 3.60. At higher pH, increasing the ionic strength of the solution can promote virus dispersion. In low conductivity solutions, retroviral glycoproteins can potentially aggregate at low pH and protect themselves from chemical damage. With the addition of 50 mM and 100 mM NaCl, all runs at all pH set points showed complete and effective clearance after 30 minutes. In summary, an operating space can be defined for effective X-MuLV inactivation, when the experiment is operated at a pH which is outside of the ASTM modular claim.

Example 5. Evaluate Existing and Redesigned Operating Conditions

[0102] A statistical design of experiment (DoE) was used to evaluate and characterize the effects of a low pH hold step for virus inactivation including the evaluation of several factors, such as protein type, pH condition, temperature, acid titrant, NaCl content, spike timing, and post-spike filtration. The DoE for the low pH hold step was used to evaluate some existing operating conditions at pH 3.70-3.75 for low pH hold. Predicted profilers including parameter estimates were generated as shown in FIG. **7**.

[0103] The DoE for the low pH hold step was also used to evaluate some existing redesigned operating conditions at pH 3.65-3.70 for low pH hold. Predicted profilers including parameter estimates were generated as shown in FIG. **8**. The redesigned operating conditions at about pH 3.65-3.70 failed to satisfy 4 LRF X-MuLV clearance at 30 minute time points at the 1.5% failure rate.

Example 6. Evaluate the Factor of Protein Types

[0104] The statistical DoE was used to evaluate and characterize the effects of a low pH hold step for virus (X-MuLV) inactivation including the evaluation of several factors, such as protein type, pH condition, temperature, acid titrant, NaCl content, spike timing, and post-spike filtration. The DoE showed statistical significance in the predictions of the multivariate models for protein types, such as the isotypes of monoclonal antibodies, but the differences between protein types, such as IgG 1 and IgG4, were not meaningful in the study ranges as shown in FIG. **9**. FIG. **9** shows scaled estimated LRF for evaluated factors, including NaCl, pH, acid titrant, temperature, protein type (mAb, monoclonal antibody), spike timing and combinations thereof. The retrospective data for existing operating conditions showed significant differences between isotypes of monoclonal antibodies, such as IgG1 and IgG4. However, these differences could be due to the differences in operating pH ranges. FIG. **9** also shows X-MuLV LRF at 30 minute time point for protein types including IgG1 and IgG2 based on retrospective data.

Example 7. Evaluate the Factor of Temperature

[0105] The statistical DoE was used to evaluate and characterize the effects of a low pH hold step for virus (X-MuLV) inactivation including the evaluation of several